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***RFLP Report***

# ALLELE FREQUENCY OF *Hin*fI RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) AND DINUCLEOTIDE REPEAT POLYMORPHISM IN THE WILMS TUMOR GENE (*WT1*) AMONG THE JAPANESE

Tadashi MATSUMOTO, Miho OKUMURA, Tomoko SAWAI, Shigeru AOKI, and Yoshiro TSUJI

*Department of Pediatrics, Nagasaki University School of Medicine, Sakamoto 1–7–1, Nagasaki 852, Japan*

The frequency of *Hin*fI RFLP and dinucleotide (CA) repeat polymorphism for the *WT1* located to chromosome 11p13 among the Japanese were estimated. Each polymorphism showed high frequency of heterozygosity and segregated independently.

***Key Words*** polymorphisms, *WT1* gene

DNA from 50 Japanese individuals (25 males and 25 females) was amplified by polymerase chain reaction (PCR) followed by *Hin*fI and/or *Dra*I digestion and electrophoresis.

*Primers for PCR.* A primer set designed by Hoban and Kelsey (1991) as WT11: 5′-GCCTGGAAGAGTTGGTCTCT-3′, and WT12: 5′-ACACAGTAATTTCAAGCAACGG-3′.

*Condition of PCR.* 100 ng of genomic DNA was amplified with 50 pmol of each primer in 50 $\mu$l PCR reaction mixture (10 mM Tris-Cl, pH 8.4/1.5 mM $MgCl_2$/50 mM KCl/250 $\mu$M of each dNTPs/3 units of Taq polymerase). Denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extention at 72°C for 2 min for 30 cycles. PCR products were electrophoresed on 6% polyacrylamide gel (PAG) in 1×TBE buffer, then stained with ethidium bromide.

1) *Hin*fI RFLP

*Polymorphic and constant DNA fragments.* A two-allele polymorphism with 240 bp (allele a) and 131 bp+109 bp (allele b), and three constant fragments (380 bp, 293 bp, and 41 bp) as described by Hoban and Kelsey (1991).

*Allele frequency.* 0.39 for allele "a" and 0.61 for allele "b". PIC=0.36. The frequency of expected and observed heterozygosity was 0.48 and 0.44, respectively.

2) CA repeat polymorphism detected after *Dra*I digestion (Fig. 1)

*Polymorphic and constant DNA fragments.* A three-allele polymorphism with

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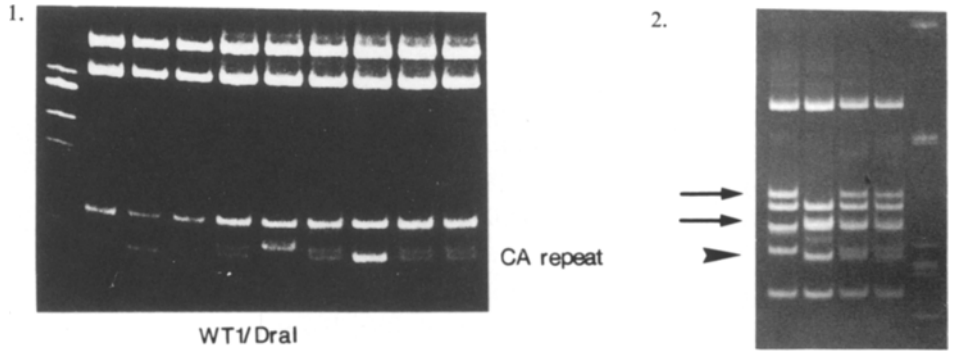


Fig. 1. CA repeats polymorphism following *Dra*I digestion. Left side lane shows rhamda phiX 174/*Hae*III size marker.

Fig. 2. *Hin*fI RFLP and CA repeats polymorphism following *Hin*fI and *Dra*I digestion. Arrows and arrow head indicate *Hin*fI RFLP and CA repeats polymorphism, respectively. Right side lane shows size marker as in Fig. 1. 40 bp and 33 bp bands are not seen in this figure.

106 bp (allele a), 104 bp (allele b), and 102 bp (allele c) and three constant fragments (409 bp, 315 bp, and 127 bp). These product length were determined in 6% PAG/8 M urea gel electrophoresis.

*Allele frequency.* 0.55 for allele "a", 0.35 for allele "b", and 0.10 for allele "c". PIC=0.45. The frequency of expected and observed heterozygosity was 0.56 and 0.31, respectively.

3) Double digestion with *Hin*fI and *Dra*I

The PCR products were digested with *Hin*fI and *Dra*I simultaneously (Fig. 2). The 109 bp *Hin*fI polymorphic band is cut to polymorphic 33 bp+constant 77 bp bands, and *Hin*fI 380 bp band is cut to constant 153 bp, 127 bp and polymorphic CA repeats bands by *Dra*I.

Polymorphic and constant fragments: 164 bp (allele "a") and 131 bp+33 bp (allele "b") *Hin*fI polymorphism and CA repeats polymorphism. Constant fragments were 293 bp, 153 bp, 127 bp, 76 bp, and 40 bp.

*Comments.* This *WT1* PCR product can be used to search two kinds of polymorphisms by two kinds of endonuclease digestion simultaneously and these two kinds of polymorphism segregate in Mendelian inheritance. As *Dra*I cut just 5′ prime of CA repeats and polymorphic fragments length are rather short, this polymorphism is easily detectable on polyacrylamide gel electrophoresis. The allele frequency of *Hin*fI RFLP in the Japanese was inverse of that in the Caucasian (Hoban and Kelsey, 1991) indicating the difference of the species. Since these polymorphisms are located within exon 10 of *WT1* and these two kinds of polymorphisms segregate independently, they are useful for the study on loss of heterozygosity (LOH) or genomic imprinting of the gene.

*Reference.* Hoban PR, Kelsey AM (1991) Nucleic Acids Res **19**: 1164